VASCULAR ADAPTATIONS TO TRANSVERSE AORTIC BANDING IN MICE

Craig J. Hartley[1], Lyssa N. Ochoa[1], Anilkumar K. Reddy[1], Lloyd H. Michael[1], Jennifer S. Pocius[1], Thuy T. Pham[1], Cederick W. Scott[1], Mark L. Entman[1], John W. Clark, Jr.[2], and George E. Taffet[1]
[1]Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, TX, USA
[2]Department of Electrical and Computer Engineering, Rice University, Houston, TX, USA

Abstract - **Transverse aortic banding in mice generates pressure overload, but cardiac hypertrophy is variable, and the effects on peripheral hemodynamics are unknown. The purpose was to characterize and model carotid and aortic blood flow patterns in banded mice using noninvasive Doppler methods. In 15 normal mice a 27-gauge needle was sutured against the transverse aorta and then removed. In 6 sham-operated mice the suture was not tied. A Doppler probe was used to measure right (R) and left (L) carotid artery (CA), aortic, and mitral blood velocity 1 day later. At 7 days the heart-weight/body-weight ratio (HW/BW) was measured. Mean aortic, mitral, and carotid velocities were similar in sham and banded mice, but peak RCA/LCA velocities were much higher in banded mice and were highly correlated to HW/BW. An esophageal Doppler probe detected high jet velocity and distal vorticity. We conclude that mice compensate for the band by increasing RCA resistance and compliance and decreasing LCA resistance to maintain normal cerebral perfusion. Velocity signals measured within one day and fitted to a lumped-parameter arterial model to estimate the pressure drop can predict the amount of cardiac hypertrophy at one week.**
Keywords - **Doppler ultrasound, carotid blood flow, cardiac hypertrophy, vascular modeling, mice**

I. Introduction

The ability to alter the genotype of the mouse has produced numerous new models to study cardiovascular function and disease processes. Many of the resulting phenotypes are subtle, and mice can often accommodate the mutations by using compensatory mechanisms to maintain blood pressure and cardiac output [1]. Thus, resting values for these and other parameters may be nearly normal, and interventions must be performed to reveal phenotypic differences in the response to stress. One of the common methods for stressing the heart in mutant mice is a pressure overload model produced by constricting the aortic arch between the origins of the carotid arteries [2]. This model (Fig 1A) is known to produce cardiac hypertrophy in normal mice (Fig. 1B-C), but it is difficult to measure the degree of stenosis (50-90%) or the pressure drop across the stenosis (10-60 mmHg) or to predict the resulting cardiac hypertrophy (0-41%). It is possible at the time of sacrifice to cannulate (and occlude) both carotid arteries to measure the pressure difference, but this act is expected to alter flow (and the pressure drop) significantly [2]. It would be desirable to have a noninvasive method which could be used at the time of surgery or shortly after which could estimate the degree of stenosis and predict the amount of hypertrophy which should occur.

The model is thought to be successful because the innominate artery which branches proximal to the band (Fig. 1A) is able to absorb some of the energy of cardiac contraction. Therefore, it is expected that the placement of the band will have profound effects on the peripheral circulation as well as on the heart. Using noninvasive Doppler methods, we have found large differences in carotid artery velocity signals measured before and after transverse aortic banding.

Therefore, the purpose of this study was to test the hypothesis that 1) ligation of the transverse aorta causes major changes in hemodynamics and elicits compensatory adaptations in the carotid arteries and cerebral circulation, 2) the changes are measurable noninvasively with Doppler ultrasound, 3) the differences in blood flow patterns in the right versus left carotid arteries are related to the degree of stenosis, 4) and the changes in velocity measured immediately after banding when combined with arterial models can estimate the pressure drop and the amount of cardiac hypertrophy which will develop after one week.

II. Methods

We employed the banding method described by Rockman [2] to produce aortic constriction in mice. Briefly, 21 mice were anesthetized using pentobarbital sodium or with a "rodent cocktail" mixture given intraperitoneally, taped supine to ECG electrodes on a temperature-controlled board [3], intubated, and placed on a respirator. The chest was then opened, the aorta was exposed and dissected free, and a suture was placed around the aortic arch between the origin of the right and left carotid arteries as shown in Fig. 1A. In 15 mice a 27 gauge (0.4 mm diameter) needle was placed against and tied to the aorta with the suture. The needle was then removed to produce a constriction with a diameter approximately equal to that of the needle. With a normal diameter

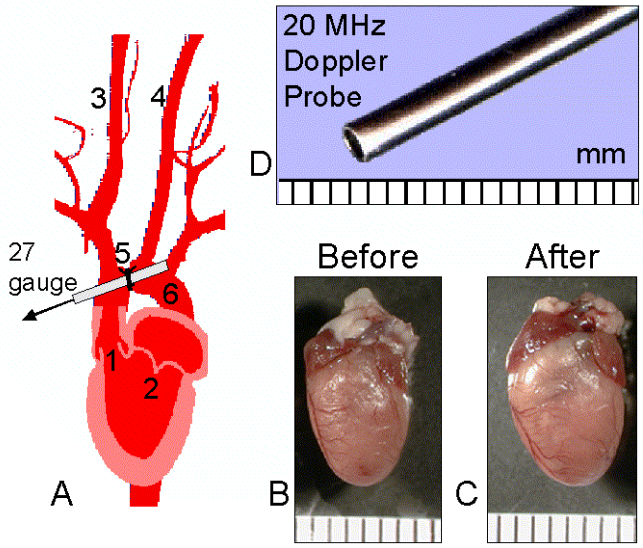

Fig. 1. Drawing of a mouse heart and great vessels (A) showing the placement of a 0.4 mm constricting band around the aortic arch to produce cardiac hypertrophy (B-C) via pressure overload. A Doppler probe (D) was used to measure flow velocity at the aortic valve (1), the mitral valve (2), the right (3) and left (4) carotid arteries, at the site of the band (5), and distal to the band (6).

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Abstract

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of 0.8 to 1.0 mm, the band produces a constriction of 75-85% by area. In 6 sham operated mice the suture was placed around the aorta but not tightened.

The mouse pulsed Doppler system was adapted from a modular instrument originally designed for use with implantable probes to measure blood flow in small vessels of dogs and rats [4,5]. For noninvasive applications in mice small hand-held probes [6] and a high-fidelity signal processor were designed [3]. The probes consist of a 1 mm diameter 20 MHz ultrasonic crystal mounted at the end of a 2 mm diameter, 10 cm long stainless steel tube as shown in Fig. 1D. An epoxy lens is molded to the front face of the crystal to focus the sound beam at a depth of 4 mm. The resulting sample volume is less than 0.02 µl (0.3 mm diameter x 0.3 mm long) at the focus. For this study we also constructed an esophageal Doppler probe by mounting a 0.5 mm Doppler at 45 degrees to the side of a 22 gauge stainless steel needle. This probe easily slides down the esophagus of a mouse and allows signals to be obtained from both carotid arteries and from multiple sites along the aorta. The esophagus lies just under the aortic arch such that all measurement sites are within 2-3 mm of the transducer face.

The Doppler signal processor is a computer based system which was designed to our specifications by Indus Instruments, Houston, TX [3]. The computer digitizes the audio Doppler signals at 125 kHz, generates a fast Fourier transform (FFT) display in real-time, and also captures and displays up to 4 other signals such as ECG. After the signals are acquired, the number of points in the FFT and the update rate can each be adjusted to optimize either temporal or frequency (velocity) resolution depending on the application. The best velocity resolution is 5 mm/s, the maximum measurable velocity is 4.6 m/s, and the best temporal resolution is 0.1 ms. A spectral envelope is then calculated generating an analog velocity waveform representing the maximum velocity within the sample volume. From the envelope signal we calculated maximum, minimum, and mean velocity at each site.

One and seven days after surgery the mice were anesthetized, taped supine to the heated ECG board and Doppler signals were taken noninvasively from both carotid arteries, the inflow and outflow tracks of the left ventricle, and where possible from the location of the aortic band and the descending aorta. In one of the banded mice signals were obtained using the esophageal probe from the left and right carotid arteries, the aortic arch at the site of the band, and immediately distal to the band in a region where flow disturbances might be expected. At seven days the animals were sacrificed and weighed, the hearts removed and weighed, and the heart-weight/body-weight ratio (HW/BW) was calculated [7]. The 15 banded mice were then divided into two groups based on HW/BW.

III. Results

Representative velocity signals from the right and left carotid arteries and from the aortic arch or stenotic jet of sham mice with no band, mice with a loose band, and mice with a tight band are shown in Fig. 2. In general, the mice with tighter bands had higher jet velocities, higher pulsatility in the right carotid artery, and lower pulsatility in the left carotid artery.

The heart-weight/body-weight ratio (mean±SEM) was 5.77±0.25 (n=6) in sham, 5.97±0.18 (n=7) in mild, and 7.35±0.33 (n=8) in severe groups as shown in Fig. 3. Mean velocities at 1 day were slightly higher (9.8) in the right carotid artery (RCA) vs the left carotid artery (LCA) (8.5) in shams and were not statistically different in banded mice. The right/left ratio (R/L) of mean velocities was 1.16±0.08 in sham, 1.18±0.13 in mild, and 1.12±0.18 in severe groups (p=ns). The ratio of maximum or peak velocities was significantly different in all groups ($p<0.05$) at 1.14±0.08 in sham, 4.40±1.0 in mild, and 6.19±0.80 in severe groups. The pulsatility index (PI) was calculated by dividing the maximum-minimum velocity by the mean velocity. PI = (max-min)/mean. The resistance index (RI) was similarly calculated by dividing the maximum-minimum velocity by the maximum velocity. RI = (max-min)/max. The RCA and LCA PI and RI were similar in shams (p=ns), but were significantly higher ($p<0.05$) in the RCA and lower in the LCA of banded mice with the highest R/L ratios in the severely banded group. Jet velocities measured transthoracically were similar at 213±32 and 235±31 cm/s in mild and severe groups compared to 90±11 cm/s in shams, but the measurements were difficult to obtain and were inconsistent because of the depth (3-5 mm) and the very small jet dimensions.

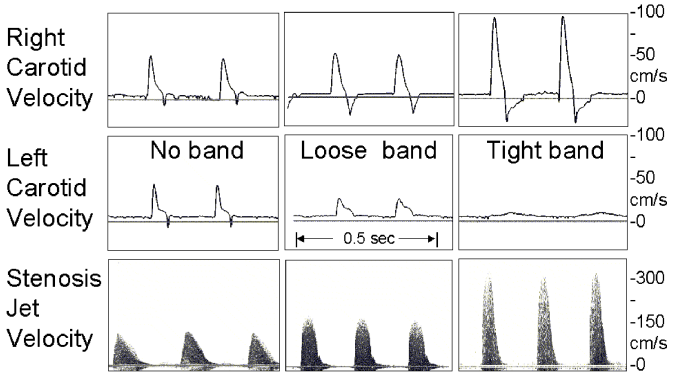

Fig. 2. Doppler velocity signals from the right and left carotid arteries and from the stenotic jet of a mouse with no band, a loose band, and a tight band.

Aortic and mitral velocity signals measured at 7 days were not different among the groups. There was a slight decrease in peak aortic velocity in the banded groups, but the differences were not

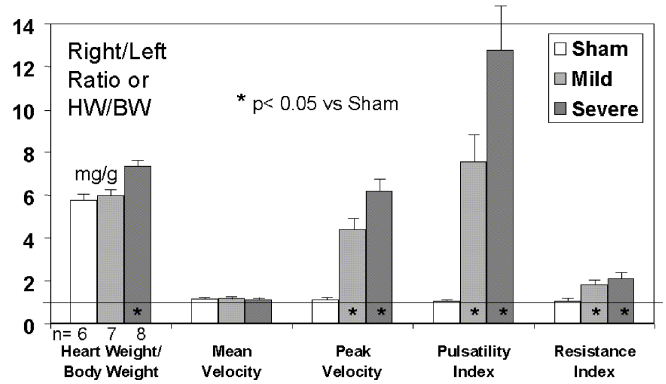

Fig. 3. Summary of results from 6 sham operated mice with no band, 7 mice with mild hypertrophy, and 8 mice with severe hypertrophy as defined by heart weight/body weight (HW/BW) ratio at 7 days. The Doppler velocity signals were taken from the right and left carotid arteries one day after surgery.

statistically significant. Peak aortic ejection velocity was 97±4.0 cm/s in the sham group, 86±4.4 in the mild group, and 93±4.4 in the severe group. Mean aortic velocity was 18.0±1.5, 18.7±0.6, and 18.9±1.2 cm/s respectively. Peak mitral filling velocity was 71±4.1, 74±4.5, and 76±5.1 cm/s, and mean mitral velocity was 14±2, 14.5±1, and 16.1±3 cm/s respectively.

Spectral Doppler signals from one of the banded mice taken with the esophageal probe are shown in Fig. 4. The right carotid artery has a pulsatility index of 11 while the left carotid artery has a pulsatility index of 2. The signal from the jet has a wide bandwidth with peak velocity of 350 cm/s which results in an estimated pressure drop ( ) P) of 49 mmHg using the simplified Bernoulli equation,) $P=4V^2$ where P is in mmHg and V is in m/s [8]. The signal from the aorta distal to the band has what appears to be a wide bandwidth signal, but when expanded as shown in Fig. 5, it is actually a narrow bandwidth signal with transient fluctuations. The frequency of the fluctuations is about 250-500 Hz and suggests a pattern of disturbed flow distal to the stenosis.

IV. Discussion

Based on studies in man [8], jet velocity would seem to be the best estimate of pressure drop across the stenosis, but using the noninvasive transthoracic probe, it was not always possible to record clean signals from the stenotic jet because of its depth, its very small size, and respiratory movements. On the other hand, good quality signals were obtained from aortic outflow, mitral inflow, and both carotid arteries of all mice.

Although the alterations in systemic hemodynamics were expected to be severe in the banded mice, we found little or no changes in mean carotid, aortic outflow, or mitral inflow velocities despite a pressure drop estimated by others to be anywhere from 10 to 60 mmHg [2]. After banding, the right carotid and subclavian arteries replace the aorta as the major source of arterial compliance resulting in a much higher peak velocity and pulsatility in the right carotid artery. These data suggest that mice compensate quickly for the right/left carotid artery pressure difference by increasing resistance in the right and decreasing resistance in the left carotid arteries to maintain cerebral perfusion at normal levels. Cardiac compensation in response to the elevated load occurs more gradually to normalize ventricular wall stress and to maintain cardiac output. We found that the amount of arterial compensation

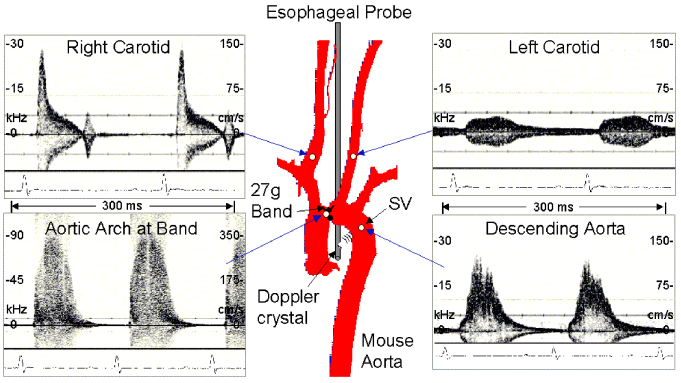

Fig. 4. Doppler signals taken at the locations shown using a 22 gauge esophageal probe in a mouse with a tight band one day after surgery.

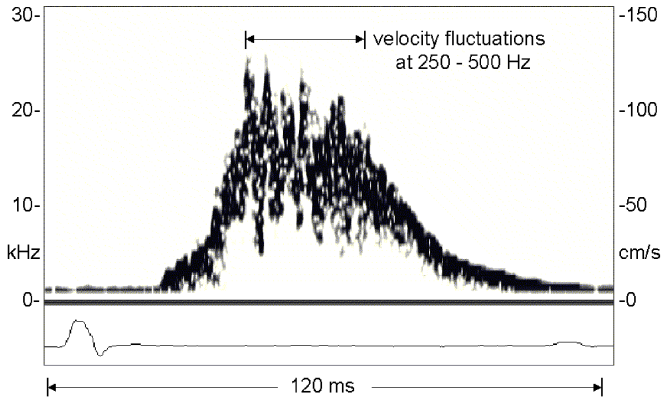

Fig. 5. Esophageal Doppler signal from tha aorta just distal to the band expanded to show transient high frequency (250-500 Hz) velocity fluctuations.

estimated by comparing right and left peak carotid velocities at one day was well correlated with (r^2=0.87) and can predict the resultant cardiac hypertrophy at one week.

Pulsatility and resistance indices are often used in patients to estimate changes in resistance and compliance distal to a velocity measurement site [8,9]. The resistance index is primarily a function of peripheral resistance while the pulsatility index relates to the compliance of the proximal vessels prior to any significant resistance. In analyzing the indices it is usually assumed that the driving pressure is "normal" and similar. This is clearly not the case here where it has been shown that the pulsatility of pressure was about twice as high in the right vs the left carotid artery after banding [2]. Thus, the changes in pulsatility in the right and left carotid arteries are due to changes in the driving pressure as well as to changes in distal vascular impedance. We found that the 12/1 difference in pulsatility in the right vs left carotid arteries of the severe group of banded mice were much higher than the 2/1 difference in pulse pressure.

In an attempt to validate the effect of the stenosis and the pressure drop on carotid flow patterns, we created a lumped parameter model of the proximal aorta and the right and left carotid arteries as shown in Fig. 6. For simplicity, the heart was modeled by a pressure source. Measurements of voltage (pressure) and current (flow) could be made anywhere with primary sites at the proximal right and left carotid arteries indicated by circles. The model was first tuned to generate realistic pressure and flow signals at the aortic root and the right and left carotid arteries as shown in the upper traces of Fig 7. Then the stenosis was added by increasing the resistance at the aortic arch (R_{aa}) by a factor of 30 (loose band) or 60 (tight band). Cerebral compensation was modeled by increasing resistance in the right carotid artery (R_{rc}) and decreasing resistance in the left carotid artery (R_{lc}) to keep the mean flows equal. In addition, the compliance of the proximal right carotid artery (C_{rc}) was increased. The resulting velocity waveforms are shown in Fig. 7. They compare favorably to the mouse velocity waveforms shown in Fig. 2. The systolic pressure drop measured in the tight model was 62 mmHg, and the mean pressure drop was 40 mmHg. The pressure pulsatility index was 0.42 at the origin of the right and 0.10 at the origin of the left carotid artery. These compare to flow pulsatility indexes of 12.4 and 0.88 respectively. Thus, the changes in flow pulsatility cannot be entirely explained by the changes in

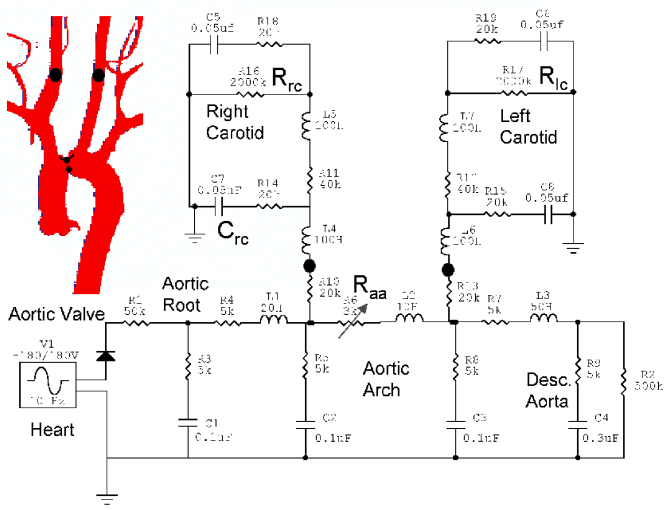

Fig. 6. Lumped parameter model of the aorta and carotid arteries of a mouse with velocity or current measurement sites at the right and left carotid arteries. The effect of the band is modeled by increasing the resistance (R_{aa}) of the aortic arch, and the resultant carotid compensation is modeled by decreasing left carotid resistance (R_{lc}) and increasing right carotid resistance (R_{rc}) and capacitance (C_{rc}).

pressure pulsatility in the model, and must be due in part to changes in vascular impedance [9].

The differences in pressure pulsatility in the model (R/L=4.2) are higher that those measured by others (R/L=2.5) [2]. However, those investigators had to occlude both carotid arteries to measure pressure, and our model would suggest that this would alter the hemodynamics and the pressure and flow waveforms considerably.

Data from the transesophageal probe shows severe constriction and generation of vorticity or turbulence. The signals in Figs. 4 and 5 in one animal show a high jet velocity (350 cm/s) with a calculated pressure drop of 49 mmHg, and the induction of high frequency velocity fluctuations distal to the band. These types of fluctuations in velocity are thought to represent vorticity, with the recorded velocity oscillating around the mean as vortices are shed and propagate through the sample volume which alternately sees the head and tail of vortices [9]. For vortex shedding to generate a narrow band signal, the sample volume has to be much smaller than the vortices, and it is encouraging to be able to detect this effect noninvasively in vessels this small.

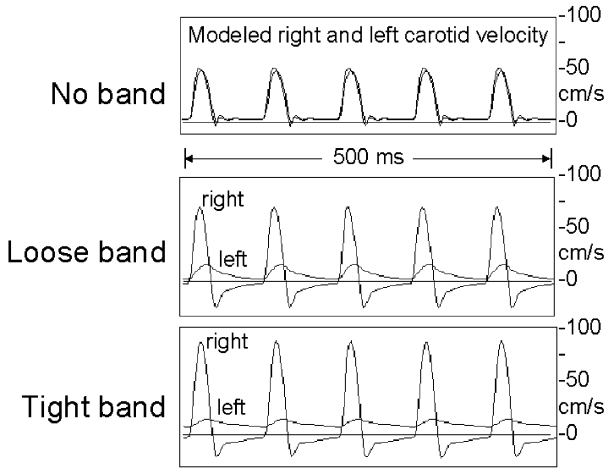

Fig. 7. Velocity waveforms from the right and left carotid arteries of the lumped parameter model with no band, a loose band, and a tight band. The model parameters were adjusted to generate waveforms matching the peak and mean values of those from mice with mild and severe hypertrophy.

V. Conclusions

We conclude that aortic banding has a large effect on peripheral vascular hemodynamics in mice. After banding, the right carotid artery replaces the aorta as the major source of arterial compliance resulting in a much higher right carotid peak velocity and pulsatility. These data suggest that mice compensate quickly by increasing resistance in the right and decreasing resistance in the left carotid artery and more slowly for the elevated load by cardiac hypertrophy to maintain cardiac output. We found that the amount of arterial compensation estimated by comparing right and left peak carotid velocities at one day was well correlated with and can predict the resultant cardiac hypertrophy at one week. We also found that modeling of the major arteries can help predict the pressure drop (which is difficult to measure) across the band.

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